Xanthine Oxidase Activity Assay Kit (Colorimetric)

Xanthine Oxidase Activity Assay Kit (Colorimetric) can be used to measure Xanthine Oxidase activity in serum and cell lysate.

Catalog number: ARG82198

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Xanthine oxidase (XO, sometimes 'XAO') is a form of xanthine oxidoreductase, a type of enzyme that generates reactive oxygen species. These enzymes catalyze the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. These enzymes play an important role in the catabolism of purines in some species, including humans.

Xanthine oxidase is defined as an enzyme activity (EC 1.17.3.2). The same protein, which in humans has the HGNC approved gene symbol XDH, can also have xanthine dehydrogenase activity (EC 1.17.1.4). Most of the protein in the liver exists in a form with xanthine dehydrogenase activity, but it can be converted to xanthine oxidase by reversible sulfhydryl oxidation or by irreversible proteolytic modification. [Provide by Wikipedia: Xanthine oxidase]

PRINCIPLE OF THE ASSAY

This Xanthine Oxidase Activity Assay Kit (Colorimetric) is a simple assay that measures the activity of xanthine oxidase in cell lysate, serum and other biological samples. This assay uses a single Working Reagent that combines the xanthine oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at O.D. 570 nm or fluorescence intensity at $\lambda ex/em$ = 530/585 nm is directly proportional to xanthine oxidase activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all reagent at-20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
HRP Enzyme	120 µL	-20°C
Xanthine (5 mM)	1.5 mL	-20°C
Dye Reagent	120 µL	-20°C
Standard (3% H_2O_2)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collection the supernatant for assay.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant for assay.

Urine: assayed directly. If particulates are present, centrifuge sample (5 minutes at 2,000 x g) and use the clear supernatant for the assay.

Cell lysate: cell samples (2 $x10^6$) can be homogenized in 100 µL of PBS. Centrifuge at 10,000 x g for 5 minutes at 4°C. Collection the supernatant for assay.

Note:

- Samples can be analyzed immediately after collection, or stored in aliquots at -20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mix 85 µL of Assay Buffer, 10 µL of 5 mM Xanthine, 1 µL of HRP Enzyme (vortex briefly before pipetting) and 1 µL of Dye Reagent.
- **Standard:** Mix 5 µL of 3% H_2O_2 and 914 µL of distilled water (final 4.8 mM) then mix 20 µL of the 4.8 mM H_2O_2 with 220 µL of distilled water to yield 400 µM H_2O_2. Dilute standards as follows:

Standard tube	H_2O_2 (µM)	Distilled water (µL)	Standard Premix, 400 µM (µL)
S1	400	0	100
S2	240	40	60
S3	120	70	30
S4	0	1000	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. During experiment, keep thawed Enzyme in a refrigerator or on ice.

COLORIMETRIC PROCEDURE

	Standard well	Sample well
Each diluted Standard	10 µL	
Each Sample		10 µL
Working Reagent	90 µL	90 µL
Tap plate to mix briefly and thoroughly. Incubate for **0 (OD_0)** and **20 minutes (OD_{20})** at **room temperature**.		
Read the absorbance at **O.D. 570 nm** (550-585 nm) for **OD_0** and **OD_{20}**		

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.01 to 2.5 U/L xanthine oxidase. Dilute the standards from **COLORIMETRIC PROCEDURE** 10X with distilled water to obtain standards at 40, 24, 12 and 0 µM H_2O_2. Final read fluorescence immediately (F_0) at λex/em = **530/585 nm**, incubate **20 minutes** at **room temperature**, and then read fluorescence again (F_{20}).

CALCULATION OF RESULTS

1. Subtract blank OD_{20} or F_{20} (distilled water, S4) from all standard OD_{20} or F_{20} values and plot the ΔOD or ΔF against standard concentrations. Determine the slope using linear regression. Calculate the ΔOD_{Sample} or ΔF_{Sample} of all samples by subtracting OD_0 or F_0 from OD_{20} or F_{20} for each sample. Do the same for the blank (distilled water, standard S4) to get ΔOD_{Blank} or ΔF_{Blank}. Calculate the activity using the equation below:

   XO Activity (U/L) = $[(\Delta R_{Sample}-\Delta R_{Blank}) / (Slope \times t)] \times n$

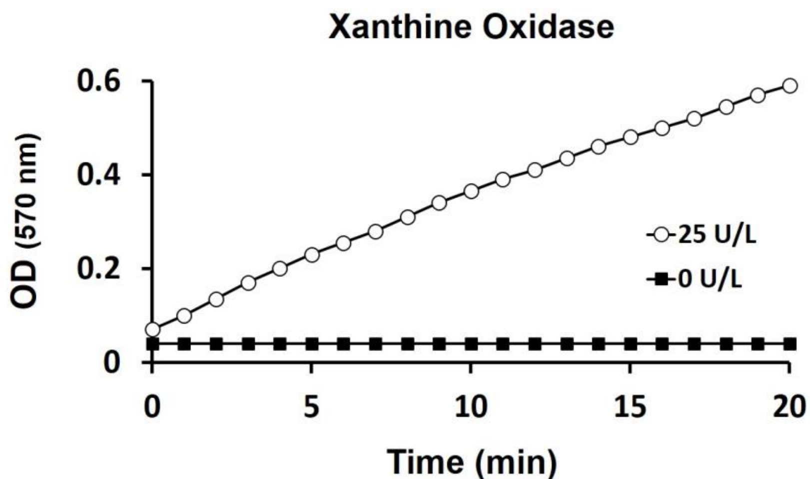
   Note:

   - ΔR_{Sample} and ΔR_{Blank}: the change in optical density or fluorescent values of the sample and blank, respectively.
   - Slope: the slope of the H2O2 standard curve.
   - t: the incubation time (20 minutes).
   - n: the dilution factor.

2. If the calculated sample XO activity is higher than 25 U/L in colorimetric assay or 2.5 U/L in fluorimetric assay, dilute sample in distilled water and repeat the assay. Multiply result by the dilution factor (n). For samples with low Xanthine Oxidase activity, the incubation time can be increased to up to 2 hours.
3. Unit definition: 1 U/L of Xanthine Oxidase catalyzes the conversion of 1 µmole of Xanthine to uric acid per minute at pH 7.0 and room temperature.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Xanthine Oxidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

OD: 0.03 U/L; FL: 0.01 U/L